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Effects of foliar spraying of L-phenylalanine and application of bio-fertilizers on growth, yield, and essential oil of hyssop [*Hyssopus officinalis* l. subsp. *Angustifolius* (Bieb.)]

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A R T I C L E I N F O

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A B S T R A C T

In order to improve growth and yield of medicinal and aromatic plants, the application of natural substances has been increased in recent years. In this study, the effects of different fertilizers under the foliar spray of L-phenylalanine on growth and physiological characteristics and essential oil content of hyssop [*Hyssopus officinalis* L. subsp. *Angustifolius* (Bieb.)], as one important medicinal and aromatic plants, at field condition in south-western Iran were investigated in a 2-year study (2016 and 2017). Experimental treatments were including arbuscular mycorrhizal fungi, chemical (N.P.K), manure, and vermicompost fertilizers with the control in the main plots and as well as three levels of the foliar application of L-phenylalanine in sub plots. Results indicated that the application of organic/biological fertilizers along with L-phenylalanine spraying have beneficial and effective role in improving the growth characteristics, shoot performance, photosynthetic pigments, and active substances of hyssop. Hence, L-phenylalanine spraying effects on improving arbuscular mycorrhizal fungi symbiosis and the application of manure and vermicompost can be a promising strategy in achieving organic production of medicinal plants such as hyssop.

1. Introduction

An increasing use of medicinal plants and their derivatives has highlighted the role of these plants in the global economic cycle, so that their consumption is not limited to developing countries and they have also become widespread in advanced countries (Van Wyk and Wink, 2017). In addition, herbs are compatible with organic cultivation practices, which also have a tendency for producers and consumers. In the production of the herbs, in addition to climatic conditions and soil factors (Bajalan et al., 2018; Moghaddam et al., 2018), the type of nutrients is of great importance, because these elements, by affecting the growth of plants, change the ratio of reproductive organs to vegetative, therefore, the quality and quantity of yield and essential oil are affected (Emami Bistgani et al., 2018; Narimani et al., 2017; Vosoughi et al., 2018).

Hyssop [*Hyssopus officinalis* L. subsp. *Angustifolius* (Bieb.)] belonging to the family Lamiaceae is a herbaceous plant of the genus *Hyssopus* native to Southern Europe, the Middle East, and the region surrounding the Caspian Sea. It is widely cultivated in some European countries such as Russia, Spain, France and Italy (Jankovský and Landa, 2002). Despite of its bitter taste, hyssop is used as a food flavoring and a variety of sauces (Kazazi et al., 2007; Wesołowska et al., 2010). The herb has many applications in the folk and modern medicine, as an expectorant, diuretic, and appetizer, and has a beneficial effect in treatment of gastrointestinal complications, laryngeal inflammation, asthma, bronchitis, herpes and accelerated wound healing (Kazazi et al., 2007). In Iranian traditional medicine (*Unani*), hyssop, known "Zoofa" in Farsi, is one of the most important medicinal plants, which the aerial of parts of

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hyssop are used for their asthma, bronchitis and cough, ulcers and wounds, carminative, antiseptic and antimicrobial (Ghasemi Pirbalouti, 2009). According to the results of previous investigations (Alinezha et al., 2013; Gayathiri et al., 2016; Kochan et al., 1999; Moro et al., 2011; Varga et al., 1998; Wang and Yang, 2010), the extracts and essential oil from *H. officinalis* have the main constituents such as quercetin, 7-*o*-bdapiofuranosyl-(1 → 2)-bdxylopyranoside, quercetin 7-o-b-dapiofuranosyl-(1 → 2)-b-dxylopyranoside 30-ob-dglucopyranoside, apigenin, luteolin, diosmin, and phenolic compounds. Some pharmacological effects of these biologically active substances including antiseptic (Mazzanti et al., 1998a), anti-bacterial (Mahboubi et al., 2011; Michalczyk et al., 2012), anti-fungal (Fraternale et al., 2004), antioxidant (Fernández López et al., 2003), cytotoxic (Renzini et al., 1999), and insecticidal (Pavela, 2004), antiviral properties, myorelaxant, antiplatelet, and α-glucosidase inhibitory activity (Alinezha et al., 2013), antihelmintic, antituberculosis activity (Hilal et al., 1978), muscle relaxing (Lu et al., 2002) and its spasmolytic action (Mazzanti et al., 1998b) were confirmed. In addition, the aerial parts of hyssop find its greatest use in flavoring preparations for alcoholic beverages, meat products and seasonings.

Considering the importance of hyssop in medicine as well as in various industries, new perspectives have been presented to researchers for improving the production and phytochemical properties of the medicinal herb (Talebi et al., 2018). Among them, the correct use of various fertilizers including vermicompost, chemical, biological, and manure by maintaining and improving soil fertility conditions, increases the quantity and quality of the hyssop. Chemical fertilizers are one of the most important supplements for plant nutrition and in recent years, the fastest way to compensate of nutrient deficiency and soil fertility (Gyaneshwar et al., 2002).

In sustainable agriculture, the applications of biological and organic fertilizers to increase soil fertility are considered as the alternative methods for chemical fertilizers (Wu et al., 2005). A lot of investigations have been done on the use of vermicompost, other organic fertilizers, and bio-fertilizers such as arbuscular mycorrhizal fungi and instead of chemical fertilizers. Hend et al. (2007) stated that this increase could be due to the balance between absorption of nutrients and water in the root environment and also, the beneficial effects of these fertilizers on vital enzymes and hormones and their stimulating effects on plant growth. In addition to the use of fertilizers, bio-stimulators can also be mentioned. These stimulants are a set of compounds that stimulate life. Some of them act as effective compounds in the plant's response to environmental conditions and others play role as growth stimulators, therefore, they can stimulate the quantitative and qualitative performance of the plant (Thomas et al., 2009). Among these compounds, amino acids like phenylalanine are organic molecules that these molecules can directly or indirectly affect the physiological activity associated with the growth and development of plants (Buchanan et al., 2000). Phenylalanine as one of the most important amino acids plays a role in the production of aromatic compounds, antioxidants.

According to the above, since the growth, development, and production of medicinal and aromatic plants are affected by genetic, ecological, and agronomic factors and their interaction effects (Bajalan et al., 2018; Emami Bistgani et al., 2018; Moghaddam et al., 2018; Narimani et al., 2017; Vosoughi et al., 2018), the selection of the optimum level of fertilizers used in each area based on environmental conditions and suitable nutrition systems are very important factors for reaching the medicinal and aromatic plants to maximum biologically active substances. Therefore, the present study was done to investigate the effect of foliar application of L-phenylalanine along application of different fertilizers on growth, photosynthetic pigments, and essential oil of hyssop.

2. Materials and methods

2.1. Plant material

This study was done in two consecutive seasons of 2016 and 2017 at the research filed of Islamic Azad University, Shahrekord Branch, Southwestern of Iran (latitude. 32° 20′ N; longitude. 50° 51′ E; altitude. 2070 m asl.). Based on the Köppen climate classification, the climate of the area of study is classified as cold, semiarid, and semi humid. Some of the meteorological parameters during the implementation of the research are presented in Table 1.

2.2. Experimental design

This study was conducted as split plot in randomized complete block design with three replications. Various treatments were inoculation of arbuscular mycorrhizal fungi [inoculation of 2 g transplant^{-1} arbuscular mycorrhizal fungi species including *Glomus* genus (*G. fasiculatum*, *G. intraradices,* and *G. mosseae*)], chemical fertilizer (N.P.K), organic fertilizers including vermicompost (10 ton ha^{-1}) and cow manure (20 ton ha^{-1}), and control (without fertilizer) in the main plots and L-phenylalanine (purchased from Merck, Germany) spray with three levels including 0, 500, and 1000 mg L^{-1} in sub plots.

2.3. Soil and fertilizer analysis

Before the experiments, soil and organic fertilizers samples were analyzed in terms of the chemical properties (Tables 2 and 3). The soil samples were air dried, crushed, and passed through a 2 mm sieve for the physiochemical analyses. The designated soil physical and chemical properties were measured, using standard methods. The soil texture determination and its particle size analysis were conducted using standard a hydrometer (Tandon, 1996); electrolytic conductivity (EC) and pH of the soil were measured on an extract of soil obtained by shaking soil with deionized water at a 1:1 (w/v) soil/water ratio by standard instruments a pH meter and conductivity meter, respectively (Janzen, 1993); organic matter by Walkley-Black (Walkley and Black, 1934) and total organic carbon by dry combustion were determined (Nelson and Sommers, 1982); total N was determined by standard Kjeldahl method (Bremner and Mulvaney, 1982); available P was measured by Olsen procedure (Olsen, 1954); exchangeable K^+ was extracted using 1 M NH^4OAc and analyzed using flamephotometry (Brown and Lilleland, 1946). Total Zn, Cu, Mn, B, and Fe were measured by atomic absorption spectroscopy (PerkinElmer, Norwalk, Conn.) after digestion of the soil with nitric and perchloric acid at 150 °C (Baker and Amacher, 1982). The cow manure and vermicompost were collected from the field farm of the Agriculture Faculty of I.A.U., Shahrekord, Iran.

2.4. Applying of treatments and maintenance

Different sources of fertilizer were mixed with soil prior to planting. The chemical fertilizers in the soil were applied at a rate of150 kg N ha^{-1} N using ammonium nitrate (33.5%), 100 kg P ha^{-1} using calcium super phosphate (15.5%), and 100 kg K ha^{-1} using potassium sulphate (48% K_2O) (150:100:100 kg ha^{-1}). In order to spraying of L-phenylalanine, its powder was dissolved in 5% acetic acid and desired concentrations were obtained with distilled water (González-Arenzana et al., 2017). The foliar application of L-phenylalanine was done at three stages of growth, including late vegetative growth, early of flowering, and 50% flowering. The L-phenylalanine solution was sprayed using an atomizer onto the leaves until it completely ran off.

2.5. Measurement of growth and physiological traits

At full flowering stage, five plants in the middle two rows were randomly selected in each plot and morphological traits, including plant

Table 1
Monthly temperature, precipitation, and average humidity during 2016 and 2017.

Month	Average temperature °C						Total precipitation mm		Average humidity %	
	minimum		mean		maximum					
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
April	−4.1	−6.6	11.9	10.3	26	25.9	40.2	74.2	52	43
May	−0.4	1.3	16.2	17	31.2	31.1	13.1	4.9	34	41
June	5.9	1.2	22.7	21.2	36.5	35.7	0.2	0	29	22
July	9.9	9.8	24.1	25.2	35.3	37.4	23.4	0	26	19

Table 2
Physicochemical properties of the soil at testing site 0–30 cm deep.

Soil texture	EC (dsm^{-1})	N (%)	Organic Carbon (%)	pH	K	P	Fe	Mn	Zn	Cu	B
					($mg.kg^{-1}$)						
Clay	1.04	0.05	0.5	7.94	238	14.8	4.89	10.47	0.55	1.27	2.49

Table 3
Chemical properties of cow manure and vermicompost used in this experiment.

	pH	EC	Cu	Mn	Fe	Zn	S	O.M	Mo	Mg	Ca	Na	K_2O	P_2O_5	N
		(dsm^{-1})	($mg.kg^{-1}$)				%								
M	8.1	3	31	155.8	701.4	102.3	0.1	76.2	38.9	0.4	1.4	1	1.2	0.5	1.5
V	8.4	1.1	–	109.6	1207.3	50.8	–	30.6	10.9	0.9	1.2	1	1	0.4	1.1

M: Cow manure, V: Vermicompost, O.M: organic matter, Mo: moisture.

height (cm), number of stems per plant, plant canopy diameter (cm), and fresh and dry weight per area land (Kg m^{-2}) were measured. The aerial parts of the herb were dried in shade during one weak. From the upper leaves of plants, the photosynthetic pigments were measured in μg. $mL^{\rightarrow 1}$, and equations (1)–(4) were used to calculate chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid, respectively (Lichtenthaler and Wellburn, 1983).

$$Chl_a = 12.25\ A_{664} - 2.79\ A_{647} \tag{1}$$

$$Chl_b = 21.21\ A_{647} - 5.1\ A_{664} \tag{2}$$

$$Chl_T = Chl_a + Chl_b \tag{3}$$

$$Cartenoids = 1000\ (A_{470}) - 2.270(Chl_a) - 81.4\ (Chl_b)\ /\ 227 \tag{4}$$

2.6. Essential oil isolation

The aerial parts of hyssop were harvested at full flowering stage (October 2016 and 2017). The tissue samples were subsequently air-dried for five days in a shaded room at 30 ± 2 °C. The collected samples were ground to fine a powder and passed through a sieve (mesh 20) to remove large pieces of debris. The essential oil of each sample was extracted from 100 g of powdered tissue by hydro-distillation method. The hydrodistillation was also performed using the Clevenger-type (made by Glass Fabricating of Ashk-e-Shishe Co., Tehran, Iran) with 500 mL water for 3 h according to the European Pharmacopoeia and then the essential oil yields were measured (Pharmacopoeia, 1988). This process was performed in triplicate at three different times. The collected essential oil was dried over anhydrous sodium sulphate and stored at 4 °C until analyzed.

2.7. Statistical analysis

The effects of treatments and their interaction were derived from two-way analysis of variance based on the GLM procedure of the SAS statistical package (SAS/STAT® v.9.2. SAS Institute Inc., Cary, NC). LSD test was used at 5% probability level to compare the mean of treatments.

3. Results

3.1. Growth characteristics

The growth characteristics of hyssop were significantly influenced by the foliar application of L-phenylalanine, as well as biological, organic and chemical fertilizers, and the results are shown in (Fig. 1 b, d, f, j, and h). As we can see, the highest plant canopy diameter was obtained in the first year in the cow manure treatment (Fig. 1 b); while in the second year; chemical fertilizer had the highest value (Fig. 1 b). In terms of number of stems, manure had the highest amounts in the first and second years, respectively (Fig. 1 d). In comparison with control, organic and chemical fertilizers increased the number of stems by 18 and 40%, respectively (Fig. 1 d). In addition, the highest plant height was observed under the applied of organic fertilizer in the first year, which was increased by 15% compared to control treatment (Fig. 1 f). In the second year (2017), in the plots with bio-fertilizer, the plant height increased by 11% compared to the control and the maximum plant height was 62 cm (Fig. 1 f).

Response of hyssop plants in terms of fresh herbal weight compared to the application of the studied fertilizers was the same in two years (Fig. 1 h). Results indicated that in the first year, vermicompost and inoculation of arbuscular mycorrhizal fungi and in the second year also these treatments had the highest biological yield (Fig. 1 h). In 2016, the applied of vermicompost and inoculation of arbuscular mycorrhizal fungi increased fresh weight. In 2017, vermicompost and inoculation of arbuscular mycorrhizal fungi increased fresh weight by 55 and 50%, respectively (Fig. 1 h). Investigating the effect of various biological, organic, and chemical fertilizers on the dry herbal weight indicated the significant results during two years. In 2016, the highest dry herbal weight under organic fertilizer which increased in contrast to the control (Fig. 1 j). On the other hand, in 2017, the highest value of dry weight was associated with organic fertilizer, which increased compared to control, although vermicompost and inoculation of arbuscular mycorrhizal fungi had no significant differences with organic fertilizer (Fig. 1

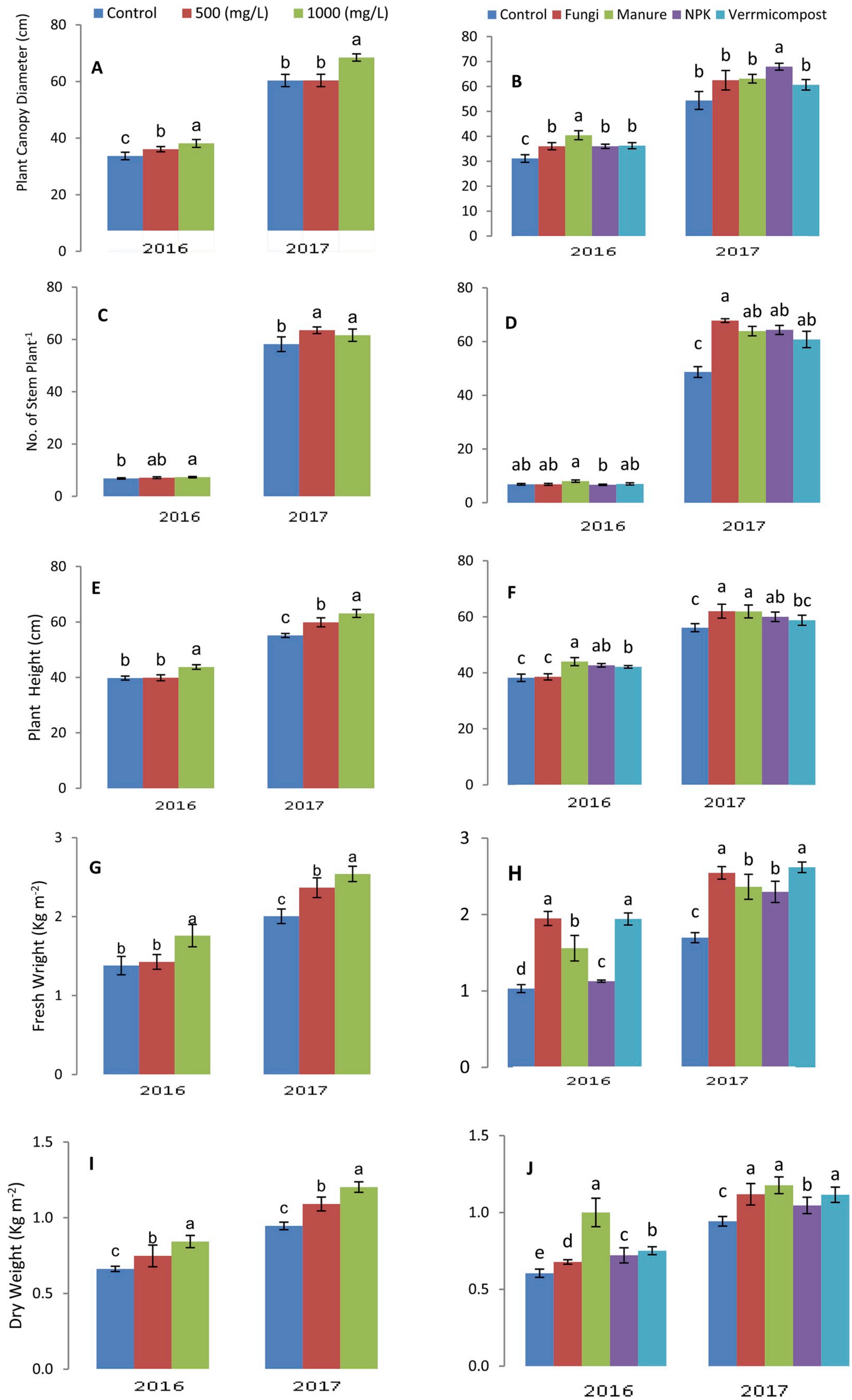

Fig. 1. Effects of L-phenylalanine (A, C, E, G, I) and application of biological, organic and chemical fertilizers (B, D, F, H, J) on plant diameter, number of stem per plant, plant height, fresh weight and dry weights of hyssop in 2016 and 2017 seasons.
All parameters are shown with standard error ($n = 3$). Different letters indicate a significant difference according to LSD test ($P \leq 0.05$).

j). It can be concluded that the addition of organic fertilizer prior to the cultivation of hyssop can play a significant role in increasing the yield of the herb.

Results of the foliar spray of L-phenylalanine for 2016 and 2017 years had significantly effects on growth and morphological characteristics (Fig. 1 a, c, e, g, and i). In the first year and in comparison with the control treatment, the foliar spray at 1000 mg L$^{\rightarrow 1}$ L-phenylalanine increased plant canopy diameter, number of stems, plant height, fresh and dry herbal weights (Fig. 1 a). In contrast, in the second year, the highest values of plant canopy diameter, number of stems, plant height, fresh and dry herbal weights were observed at 1000 mg L$^{\rightarrow 1}$ L-phenylalanine (Fig. 1 a, c, e, g, and i). In addition, there was a significant difference between 500 mg L$^{\rightarrow 1}$ L-phenylalanine with control in all vegetative traits (except for the number of stems in the first year) in each two years of study.

The interaction effects of L-phenylalanine and fertilizers on the studied characteristics are presented in Table 4. In both years of evaluation, at 500 mg L$^{\rightarrow 1}$ L L-phenylalanine, the best fertilizer for combination was arbuscular mycorrhizal fungi to increment of plant canopy diameter, which increased it by 25% at first year and 39% at second year in comparison to control (Table 4). In contrast, the foliar spray of L-phenylalanine (1000 mg L$^{\rightarrow 1}$) in combination with organic and chemical fertilizers had the best effect on plant canopy diameter in 2016 and 2017 years, respectively (Table 4). In 2016, the best treatment to improve the number of stems was foliar application of L-phenylalanine along with the applied of organic fertilizer, so that the foliar application of 500 and 1000 mg L$^{\rightarrow 1}$ L-phenylalanine increased in comparison with control (Table 4). On the other hand, in 2017, the highest number of stems was related at 500 mg L$^{\rightarrow 1}$ L-phenylalanine under the applied vermicompost. In the foliar application at 1000 mg L$^{\rightarrow 1}$, its combination with biological and organic fertilizers was the best treatment for increasing the number of stems of hyssop (Table 4). Assessment of hyssop plant reactions to the simultaneous L-phenylalanine spraying and fertilizer application in terms of plant height in average of two years indicated that application of manure is the best way to improve this trait and increase it by 25% compared to control. In 2016, 2017, the highest plant heights were obtained from the plants sprayed what 1000 mg L$^{\rightarrow 1}$ L-phenylalanine under the applied organic and biological fertilizer (Table 4). For biomass yield, results include the foliar application at 500 mg L$^{\rightarrow 1}$ L-phenylalanine and plants inoculated with arbuscular mycorrhizal fungi had the highest values for this trait for 2016 and 2017 years (Table 4). On the other hand, the simultaneous use of L-phenylalanine (1000 mg L$^{\rightarrow 1}$) with biological and organic fertilizers (at 2016 year), and foliar spray L-phenylalanine (1000 mg L$^{\rightarrow 1}$) under the applied vermicompost (at 2017 year) had significant effects on fresh herbal weight. For dry herbal weight, the effect of L-phenylalanine × fertilizer in both years and for L-phenylalanine concentrations approximately was same, and the combined treatment of L-phenylalanine and organic fertilizer had the highest amounts of dry weight. In total, 1000 mg L$^{\rightarrow 1}$ L-phenylalanine under the applied organic fertilizer in both years increased dry weight of hyssop (Table 4).

Results of this study on effect of year on growth parameters indicated that the all measurement features including plant height, number of stems per plant, plant canopy diameter, and fresh and dry weight (per area land) were significantly affected by the year. Indeed, in second year reached to the highest levels these parameters. For example, the highest fresh and dry herbal weights were obtained from second experimental (2.8 and 1.4 kg m^{-2}, respectively) in the foliar application of L-phenylalanine under the applied organic and vermicompost fertilizers treatments compared to first experimental year (2.2 and 1.0 kg m^{-2}, respectively) in the same treatments.

3.2. Photosynthesis pigments

Assessment of main effects in the present study suggests a roughly similar trend on photosynthetic pigments (Fig. 2 a-h). Accordingly, the highest amount of chlorophyll *a* in the both years was observed in arbuscular mycorrhizal fungi treatment, which increased 15 and 34%, respectively (Fig. 2 b). It should be noted that in the second year, the application of chemical fertilizer that improved concentration of chlorophyll *a* by 31% had not any significantly differences with fungal fertilizers (Fig. 2 b). Similar results were obtained for chlorophyll *b* and total chlorophyll (Fig. 2 d and f). In other words, using fungal fertilizers increased them by 24%, followed by chemical fertilizer, which had a significant effect on increasing the photosynthetic pigments concentration. Carotenoid reaction to studied factors showed that its highest level was observed in vermicompost fertilizer and manure in the first year. The results of the second year also showed that in the experimental units containing manure, the highest concentration of carotenoids was observed (Fig. 2 h).

Table 4
Effect of foliar application of L-phenylalanine and the application of biological, organic and chemical fertilizers on growth characters of hyssop in 2016 and 2017.

Treatments	Plant Canopy Diameter (cm)		No. Of Stem Plant^{-1}		Plant Height (cm)		Fresh Weight (Kg m^{-2})		Dry Weight (Kg m^{-2})	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
L-phenylalanine 0 (mg/L)										
Control	26.22 ± 0.55	55.67 ± 1.64	6.44 ± 0.59	44.33 ± 2.19	36.33 ± 1.45	53.33 ± 0.88	0.83 ± 0.01	1.63 ± 0.12	0.55 ± 0.02	0.89 ± 0.02
AMF[a]	31 ± 0.51	48 ± 2.02	6.44 ± 0.68	69.33 ± 0.73	39 ± 1.53	56 ± 1.73	1.62 ± 0.06	2.32 ± 0.03	0.73 ± 0.01	0.94 ± 0.04
Manure	36.22 ± 1.54	65.33 ± 1.3	7.67 ± 0.96	59 ± 3.06	39.67 ± 0.88	54.67 ± 1.45	1.21 ± 0.03	1.74 ± 0.08	0.65 ± 0.02	1.03 ± 0.03
NPK	34.56 ± 0.87	68.67 ± 2.4	6.44 ± 0.29	68.5 ± 2.25	41.67 ± 0.88	53.67 ± 0.88	1.16 ± 0.03	1.87 ± 0.04	0.7 ± 0.01	0.84 ± 0.02
Vermicompost	40.44 ± 0.87	64 ± 3.01	7.11 ± 0.62	49.67 ± 2.95	42 ± 1.15	58 ± 2.08	2.08 ± 0.05	2.46 ± 0.07	0.68 ± 0.01	1.03 ± 0.06
L-phenylalanine 500 (mg/L)										
Control	31.56 ± 2.08	52.67 ± 1.59	7.34 ± 0.67	55.67 ± 0.88	35.33 ± 0.88	54 ± 2.31	1.12 ± 0.05	1.55 ± 0.04	0.56 ± 0.02	0.89 ± 0.04
AMF	39.55 ± 0.91	73.17 ± 2.4	6.56 ± 0.68	65.67 ± 1.17	35 ± 0.58	58.67 ± 1.76	2.01 ± 0.05	2.82 ± 0.07	0.63 ± 0.01	1.04 ± 0.04
Manure	37.89 ± 0.97	56.67 ± 0.83	8.34 ± 0.88	64.5 ± 1.76	43.33 ± 0.88	68.33 ± 1.86	1.24 ± 0.03	2.59 ± 0.07	1.03 ± 0.03	1.37 ± 0.04
NPK	36.11 ± 2.08	65.83 ± 1.36	6.67 ± 0.51	65 ± 2.89	43.67 ± 0.88	64 ± 1.53	1.13 ± 0.01	2.23 ± 0.08	0.56 ± 0.01	1.12 ± 0.04
Vermicompost	35.33 ± 1.07	53.5 ± 0.76	6.67 ± 0.7	66.67 ± 2.13	42 ± 0.58	54.33 ± 1.2	1.63 ± 0.02	2.64 ± 0.11	0.72 ± 0.01	1.03 ± 0.04
L-phenylalanine 1000 (mg/L)										
Control	35.56 ± 1.28	54.83 ± 2.03	6.78 ± 0.11	46 ± 2.25	43 ± 0.58	61 ± 0.58	1.14 ± 0.02	1.91 ± 0.04	0.71 ± 0.01	1.05 ± 0.02
AMF	37.55 ± 1.87	66.33 ± 1.67	7.56 ± 0.48	68.33 ± 0.67	41.67 ± 0.88	71.33 ± 1.2	2.02 ± 0.06	2.49 ± 0.1	0.67 ± 0	1.38 ± 0.02
Manure	47.22 ± 1.16	67.33 ± 1.3	8.11 ± 0.59	68.17 ± 2.03	49 ± 1.15	62.67 ± 1.76	2.03 ± 0.01	2.75 ± 0.13	1.04 ± 0.03	1.12 ± 0.05
NPK	37.33 ± 1.39	69.33 ± 3.48	6.89 ± 0.4	59.5 ± 0.5	42.67 ± 1.45	62.33 ± 0.33	1.1 ± 0.02	2.79 ± 0.09	0.9 ± 0.02	1.17 ± 0.02
Vermicompost	33 ± 1.15	64.5 ± 2.47	7.22 ± 1.09	66 ± 2.65	42.33 ± 0.88	64 ± 3.06	2.11 ± 0.01	2.76 ± 0.13	0.85 ± 0.02	1.28 ± 0.07
LSD 5%	1.46	2.87	0.46	2.98	1.29	2.25	0.05	0.10	0.02	0.05

[a] Arbuscular Mycorrhizal Fungi.

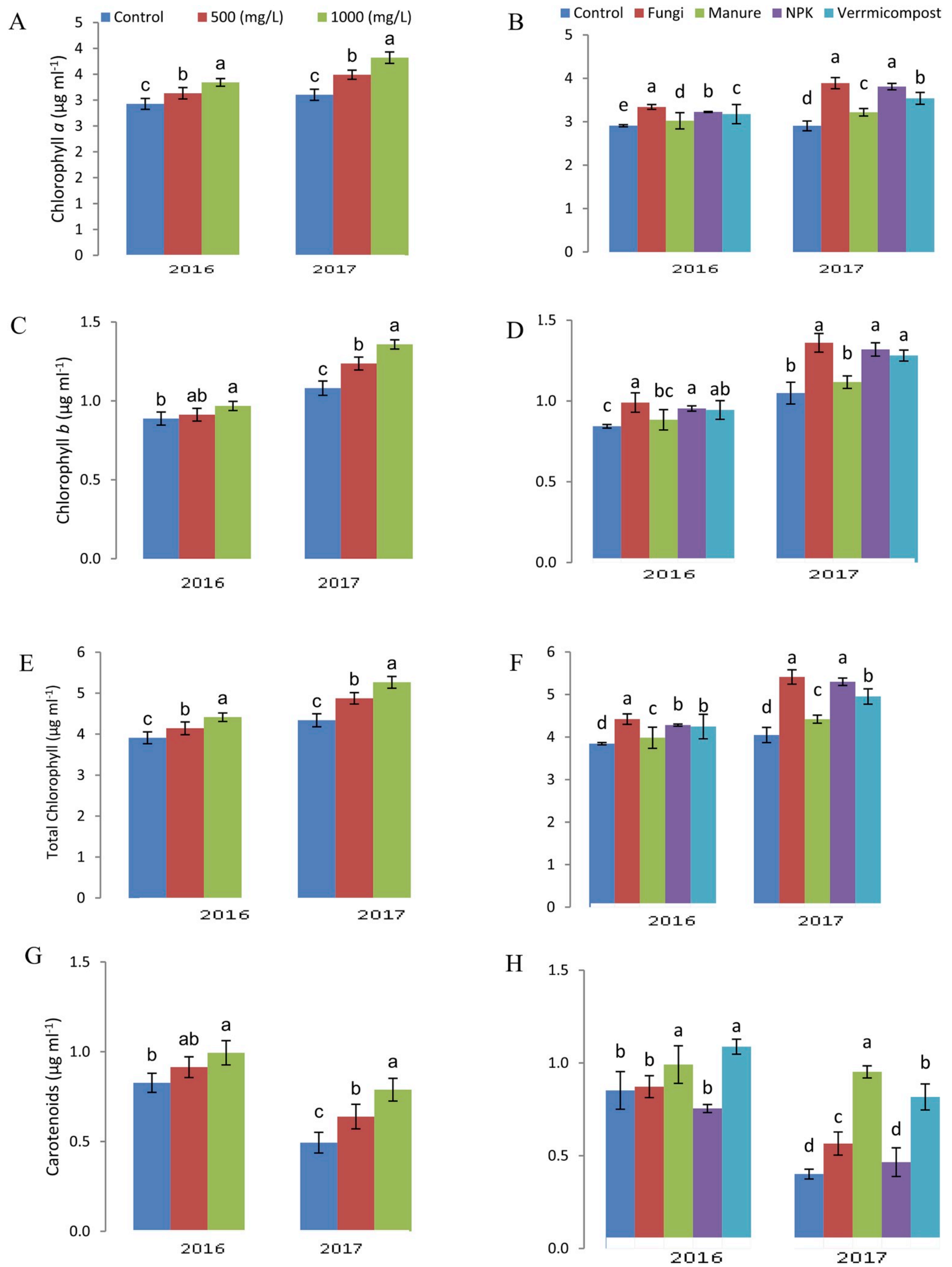

Fig. 2. Effects of L-phenylalanine spraying (A, C, E, G) and application of biological, organic and chemical fertilizers (B, D, F, H) on chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid in the leaves hyssop in 2016 and 2017.
All parameters are shown with standard error ($n = 3$). Different letters indicate a significant difference according to LSD test ($P \leq 0.05$).

Regarding the application of L-phenylalanine in the three levels (Fig. 1 a, c, e, and g), the results were quite similar. In both years, the highest photosynthetic pigments were observed in the plots with L-phenylalanine (1000 mg L^{-1}), so increased chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid (Fig. 1 a, c, e, and g). It should be noted that in the majority of cases, there was a significant difference among foliar spray at L-phenylalanine (500 mg L^{-1}) and control.

Results of L-phenylalanine × fertilizer (Table 5) in two years had significantly effects on amounts of chlorophyll *a*, chlorophyll *b*, and total chlorophyll. The highest values of chlorophylls were obtained from the combination of L-phenylalanine (500 mg L^{-1}) + vermicompost for 2016, while for 2017 the highest concentrations of photosynthesis pigments were obtained from the combination of L-phenylalanine (500 mg L^{-1}) × arbuscular mycorrhizal fungi and L-phenylalanine (500 mg L^{-1}) × chemical fertilizer.

Results of this study indicated that there were not differences among two experimental years for chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid contents (Table 5).

3.3. Essential oil content

The essential oils extracted from the aerial parts of hyssop produced a clear, yellow liquid. The essential oil yields were obtained from harvested herbs at 2016 lower than and harvested hyssop at 2017 (Fig. 3 a-b). Results of this study indicated that there were significant differences between different fertilizers for essential oil content in both years (Fig. 3

Table 5
Effect of foliar application of L-phenylalanine and the application of biological, organic and chemical fertilizers on photosynthesis pigments of hyssop in 2016 and 2017.

Treatments	Chlorophyll *a* (μg ml^{-1})		Chlorophyll *b* (μg ml^{-1})		Total Chlorophyll (μg ml^{-1})		Carotenoids (μg ml^{-1})	
	2016	2017	2016	2017	2016	2017	2016	2017
L-phenylalanine 0 (mg/L)								
Control	2.95 ± 0.04	2.52 ± 0.14	0.84 ± 0.02	0.83 ± 0.07	3.88 ± 0.04	3.42 ± 0.21	0.71 ± 0.05	0.43 ± 0.02
AMF[a]	3.45 ± 0.05	3.51 ± 0.02	1.15 ± 0.05	1.14 ± 0.01	4.73 ± 0.08	4.86 ± 0.06	0.86 ± 0.04	0.38 ± 0.02
Manure	2.69 ± 0.06	2.9 ± 0.03	0.75 ± 0.05	1.04 ± 0.06	3.53 ± 0.1	4.11 ± 0.04	0.61 ± 0.02	0.86 ± 0.01
NPK	3.21 ± 0.01	3.55 ± 0.04	0.94 ± 0.02	1.24 ± 0.11	4.22 ± 0.02	5.03 ± 0.08	0.8 ± 0.04	0.23 ± 0.02
Vermicompost	2.33 ± 0.02	3.04 ± 0.04	0.76 ± 0.03	1.16 ± 0.01	3.19 ± 0.03	4.27 ± 0.05	1.16 ± 0.08	0.57 ± 0.04
L-phenylalanine 500 (mg/L)								
Control	2.85 ± 0.04	2.96 ± 0.03	0.86 ± 0.02	1.07 ± 0.07	3.82 ± 0.07	4.15 ± 0.02	0.63 ± 0.03	0.38 ± 0.04
AMF	3.13 ± 0.04	3.79 ± 0.04	0.8 ± 0.08	1.41 ± 0.02	3.98 ± 0.07	5.37 ± 0.03	1.05 ± 0.1	0.54 ± 0.07
Manure	2.61 ± 0.03	3.27 ± 0.01	0.79 ± 0.06	1.07 ± 0.03	3.46 ± 0.08	4.39 ± 0	1.14 ± 0.07	0.98 ± 0.01
NPK	3.24 ± 0.02	3.82 ± 0.03	0.97 ± 0.02	1.35 ± 0.03	4.32 ± 0.03	5.36 ± 0.14	0.73 ± 0.03	0.42 ± 0.02
Vermicompost	3.82 ± 0.02	3.61 ± 0.05	1.15 ± 0.03	1.28 ± 0.03	5.14 ± 0.11	5.1 ± 0.01	1.02 ± 0.06	0.88 ± 0.09
L-phenylalanine 1000 (mg/L)								
Control	2.92 ± 0.03	3.23 ± 0.05	0.83 ± 0.02	1.25 ± 0.02	3.83 ± 0.03	4.57 ± 0.02	1.22 ± 0.13	0.4 ± 0.08
AMF	3.43 ± 0.03	4.37 ± 0.04	1.03 ± 0.05	1.53 ± 0.01	4.55 ± 0.13	6 ± 0.07	0.71 ± 0.01	0.78 ± 0.04
Manure	3.76 ± 0.02	3.48 ± 0.04	1.11 ± 0.03	1.25 ± 0.02	4.97 ± 0.01	4.76 ± 0.04	1.23 ± 0.08	1.02 ± 0.07
NPK	3.22 ± 0.03	4.05 ± 0.03	0.95 ± 0.05	1.37 ± 0.04	4.3 ± 0.06	5.51 ± 0.06	0.74 ± 0.04	0.75 ± 0.04
Vermicompost	3.37 ± 0.04	3.97 ± 0.01	0.92 ± 0.03	1.4 ± 0.01	4.42 ± 0.11	5.49 ± 0.08	1.08 ± 0.07	1 ± 0.03
LSD 5%	0.05	0.07	0.06	0.06	0.10	0.11	0.09	0.07

[a] Arbuscular Mycorrhizal Fungi.

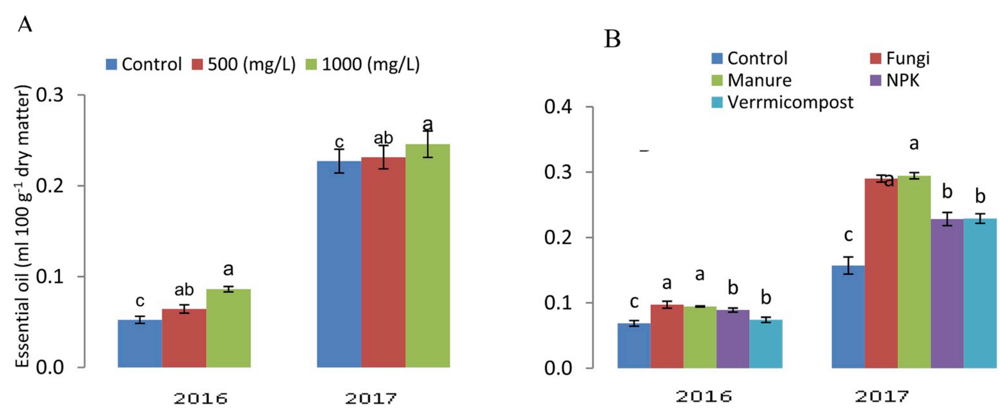

Fig. 3. Effects of L-phenylalanine spraying and the application of biological, organic and chemical fertilizers on essential oil content of hyssop in 2016 and 2017. All parameters are shown with standard error ($n = 3$). Different letters indicate a significant difference according to LSD test ($P \leq 0.05$).

b). The foliar application of L-phenylalanine on essential oil yield in two years had an increasing trend. Results in this study indicated the foliar spray L-phenylalanine increased quantity yield of the essential oil from hyssop (Fig. 3 a). The interaction effects of L-phenylalanine and fertilizers on essential oil content are presented in Table 6. In both years of evaluation, at 500 mg L$^{\rightarrow 1}$ L-phenylalanine, the best fertilizers for combination were arbuscular mycorrhizal fungi, vermicompost and manure to increment of essential oil content, which increased it by 40% at first year and 80% at second year in comparison to control.

Results of this study on effect of year on the essential oil showed that the essential oil yield was significantly affected by the year the maximum essential oil yield was obtained from second experimental (0.39 mL/100 g dry matter) in the treatment of the foliar application of L-phenylalanine (500 mg L$^{\rightarrow 1}$) under the applied manure fertilizer compared to first experimental year (0.11 mL/100 g dry matter) in the foliar application of L-phenylalanine (1000 mg L$^{\rightarrow 1}$) under the applied manure fertilizer.

4. Discussion

Results of fertilizer treatments in this study indicated that in both years, the best selection for increasing growth traits (plant canopy diameter, plant height, number of stems plant$^{\rightarrow 1}$, and fresh and dry herbal weights) was to use organic and biological fertilizers (Fig. 1 b, d, f, j, and h). In the first year, the highest values for plant canopy diameter and number of stems plant$^{\rightarrow 1}$ were observed in the applied organic fertilizer, whereas, in the second year, the highest values were obtained from the herbs inoculated with arbuscular mycorrhizal fungi (Fig. 1 b and d). Joshi et al. (2015) reported that vermicompost is ideal organic manure or biological fertilizer for better growth and yield of many plants. Vermicompost and manure fertilizers can increase the production of horticultural crops and prevent them from harmful pests without polluting the environment. Many investigators have argued that herbs cultivated in organic farming system, due to improved soil quality, often higher contain vitamin B and C, phenolic compounds, and secondary metabolites than medicinal and aromatic plants grown in conventional farming systems (Sharma, 2001). Shahmohammadi et al. (2014) reported the highest biological yield, seed yield were obtained in bio-fertilizer treatments and also, the application of 10 ton ha$^{\rightarrow 1}$ bio--fertilizer + compost had the greatest effect on yield of fruits.

The foliar application of various concentrations of L-phenylalanine on growth traits in two years had an increasing trend. In all studied

Table 6
Effect of foliar application of L-phenylalanine and the application of biological, organic and chemical fertilizers on essential oil content of hyssop in 2016 and 2017.

Treatments	Essential oil content (ml 100 g^{-1} dry matter)	
	2016	2017
L-phenylalanine 0 (mg/L)		
Control	0.06 ± 0.007	0.15 ± 0.012
AMF[a]	0.10 ± 0.009	0.30 ± 0.011
Manure	0.08 ± 0.001	0.29 ± 0.0130
NPK	0.09 ± 0.006	0.21 ± 0.007
Vermicompost	0.08 ± 0.002	0.25 ± 0.011
L-phenylalanine 500 (mg/L)		
Control	0.06 ± 0.008	0.18 ± 0.038]
AMF	0.10 ± 0.012	0.33 ± 0.0142
Manure	0.08 ± 0.001	0.39 ± 0.006
NPK	0.08 ± 0.005	0.23 ± 0.012
Vermicompost	0.10 ± 0.001	0.33 ± 0.011
L-phenylalanine 1000 (mg/L)		
Control	0.08 ± 0.003	0.15 ± 0.012
AMF	0.08 ± 0.005	0.23 ± 0.0003
Manure	0.08 ± 0.001	0.31 ± 0.006
NPK	0.09 ± 0.004	0.24 ± 0.03
Vermicompost	0.13 ± 0.008	0.21 ± 0.007
LSD 5%	0.01	0.03

[a] Arbuscular Mycorrhizal Fungi.

morphological traits, the highest values were observed at 1000 mg L^{-1} L-phenylalanine, and its difference with control treatment and also spraying at 500 mg L^{-1} (except for stem number) was statistically significant (Fig. 1 a, c, e, g, and i). Similarity, El-Din and El-Wahed (2005) stated that phenylalanine treatment significantly increased growth characteristics such as height, number of branches, number of flower heads and fresh and dry weights of aerial part in chamomile and fennel (El-Din and El-Wahed, 2005). Results of an investigation (Reham et al., 2016) showed that the foliar application of phenylalanine (100 ppm) had significantly increasing in the growth parameters of Genovese basil. In addition, phenylalanine application significantly increased fresh and dry weight of some *Datura* species during vegetative and flowering stages (Youssef et al., 2004).

The interaction effects of L-phenylalanine and fertilizers on growth parameters indicated that L-phenylalanine at 500 mg L^{-1} under inoculation of the plants with arbuscular mycorrhizal fungi had higher impacts in comparison to control (Table 4). Generally, the highest values plant height, canopy diameter, fresh and dry herbal weights were obtained from the hyssop plants sprayed what 1000 mg L^{-1} L-phenylalanine under the applied organic and biological fertilizer. Results of a study (Hadi and Ghale, 2016) indicated the application of vermicompost at a rate of 12 ton ha^{-1} and foliar application of amino acid at the flowering stage had positive and significant effects on dry flower yield and chamazulen yield in chamomile (*Matricaria chamomilla* L.). Results of an investigation (Abdollahi et al., 2017) indicated that the foliar application polyamines such as putrescine (1 mM) along with 40–60% vermicompost substitution can improve growth characteristics and yield of crops.

According to results of the interaction effects of L-phenylalanine × fertilizer in the first year, L-phenylalanine (500 mg L^{-1}) + vermicompost combined showed the highest amounts of chlorophyll *a*, chlorophyll *b*, and total chlorophyll (Table 5). The facilitating resource acquisition such as nitrogen, phosphorus, and other essential minerals (Amooaghaie and Golmohammadi, 2017) in vermicompost-treated plants probably has contributed to increasing leaf carotenoid content that resulted in the increase of the photosynthetic efficiency. In 2017, the highest values of chlorophylls were in the combination of L-phenylalanine (500 mg L^{-1}) × arbuscular mycorrhizal fungi and L-phenylalanine (500 mg L^{-1}) × chemical fertilizer. In the same way, the highest increase in chlorophyll *a*, chlorophyll *b*, and total chlorophyll in the first year was observed in experimental units with L-phenylalanine (1000 mg L^{-1}) + manure; while their increment in the second year was related to combination of L-phenylalanine at 1000 mg L^{-1} with inoculation of arbuscular mycorrhizal fungi. In the case of carotenoids, if manure along with L-phenylalanine was used in one plot, we saw the highest amounts of this photosynthetic pigment in both years.

Amino acids play a role in biosynthesis of organic compounds such as pigments, vitamins, alkaloids, enzymes, terpenoids, coenzymes, purine, and pyrimidine bases (Wu, 2009). As a result, after absorption of biological stimulators (with amino acids), the plant can act in the shortest time without any stress or performance loss (du Jardin, 2015). In another study (Rashad et al., 2002), the application of L-phenylalanine (250 mg L^{-1}) had the significant effect on growth parameters, photosynthetic pigments, and capsaicin levels in pepper (*Capsicum annum* L.). In this study, significant increase was observed in the use of L-phenylalanine by improving the growth and development of the hyssop plants, is related to increment of water and nutrients and absorption consequently enhancing photosynthesis, which improved vegetative traits such as plant height and dry weight of the plant (Dahab and El-Aziz, 2006).

The differences in the essential oil yields among the plant samples harvested in 2016 and 2017 years could be attributed to plant age, moisture conditions, temperature, and improvement effects of organic and biological fertilizers on growth and morpho-physological parameters such as photosynthetic pigments, total dry weight and leaf oil gland population at second year study (Ghasemi Pirbalouti et al., 2013). Previous investigations (Emami Bistgani et al., 2016, 2018; Salehi et al., 2018; Zardak et al., 2018) increase of biomass and oil yields of Iranian herbs under biological and organic fertilizers in compare with control and chemical fertilizer. According results of effects fertilizers on the oil yield in this study, the highest the essential oil yields were obtained from organic manure and arbuscular mycorrhizal fungi. Our results are in agreement with previous investigations that found higher essential oil percentage in herbs due to application of organic and biological fertilizers as compared to control plants (Emami Bistgani et al., 2018; Javanmardi and Ghorbani, 2012; Salehi et al., 2018; Zardak et al., 2018). The enhance in essential oil yield in treatments of organic and biological fertilizers may be due the higher supply of Nitrogen from these fertilizers leading to higher activity of photosynthesis pigments (chlorophyll) and secondary metabolites, such as essential oil (Emami Bistgani et al., 2018). In addition, the highest essential oil yields were observed from the foliar spray of L-phenylalanine (500 and 1000 mg L^{-1}), and their difference with control were statistically significant. Similarity, EL-Zefzafy et al. (2016) showed that phenylalanine spraying on *Artemisia abrotanum* aerial parts significantly increased the essential oil content and yield by increasing the concentration of phenylalanine (EL-Zefzafy et al., 2016). L-Phenylalanine spraying increased essential oil content, possibly it acted as a potent inducer for increasing the biosynthesis of secondary metabolites. Amino acids such as phenylalanine function in the synthesis of terpenoids and volatile compounds, etc. It is clear that more than one biosynthetic route is involved in the conversion of phenylalanine into plant volatiles (Gonda et al., 2018; Vogt, 2010).

In conclusion, the present study is apparently the first report of morphological, physiological and essential oil responses of hyssop to different fertilizers and L-phenylalanine spraying. In general, the results indicated that the studied characteristics were obtained in L-phenylalanine spraying under the applied biological and organic fertilizers, including arbuscular mycorrhizal fungi, vermicompost and manure. Positive effects of the application of L-phenylalanine along biological and organic fertilizers can be attributed to their potentials in the supply of nutrients, as a result of enhancement of photosynthesis, and also the close and positive relationship often observed between pure photosynthesis and the amount of mineral nutrients in green leaves. Finally, the results of this study indicated that the quantitative and qualitative performances of hyssop were influenced by the foliar application of L-phenylalanine and the soil applied of vermicompost, manure, and inoculation of arbuscular mycorrhizal fungi. Therefore, it can be concluded that with using biological stimulators such as L-phenylalanine, the ability of produce medicinal metabolites can be increased and in the direction of sustainable agriculture and reduction of biological contamination, an effective step was taken.

Results of this study indicated that year significantly affected on growth parameters and essential oil yield. Indeed, in second year reached to the highest levels growth parameters and essential oil yield. It is seem that due to the application of the organic fertilizers which takes a longer time to show effect, hence resulting in much better performance in the second year.

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Appendix A. Supplementary data

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